FINAL REPORT

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Principal Investigator	Professor Ralph Mitchell Division of Engineering and Applied Sciences Harvard University
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We studied the microbial degradation of polyurethane top-coatings in the bscence of Cr(VI). We have shown, using electrochemical impedance spectroscopy (EIS), that these polymers degrade in the presence of the ambient microflora. As a next step in our investigation, we initiated enrichment cultures in order to isolate and cultivate microorganisms capable of utilizing polyurethane as a sole source of carbon and energy. Degradation of polyurethane coating polymers was achieved after four months of incubation in one of our cultures incubated with microorganisms commonly found in the ambient environment. Two bacteria were isolated from one of our enrichment cultures. They were characterized both biochemically and genetically.

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OBJECTIVES

We initiated this research project in March 1998. It had two primary objectives. The first was to investigate degradation of top coatings and protective primers in the absence of chromium. The second objective was to test the use of biocides to protect coatings against biodegradation, and to assess the effects of alternative anti-corrosives, such as molybdenum, on the microbial degradation of the protective polymers.

ACCOMPLISHMENTS

We studied the microbial degradation of polyurethane top-coatings in the absence of Cr(VI). We have shown, using electrochemical impedance spectroscopy (EIS), that these polymers degrade in the presence of the ambient microflora. As a next step in our investigation, we initiated enrichment cultures in order to isolate and cultivate microorganisms capable of utilizing polyurethane as a sole source of carbon and energy. Degradation of polyurethane coating polymers was achieved after four months of incubation in one of our cultures incubated with microorganisms commonly found in the ambient environment. Two bacteria were isolated from one of our enrichment cultures. They were characterized both biochemically and genetically.

We monitored the degradability of primers and top-coatings using EIS to evaluate the degradative process at the polymer-metal interface. A microorganism resistant to Cr(VI) was detected and identified as *Pseudomonas aeruginosa*, a common soil bacterium.

We also initiated an investigation into the microflora capable of degrading polyurethane coatings. In the culture medium, polyurethane coatings (Bayer Co., Pittsburgh, PA) were added as the sole source of carbon and energy. The minimum salt solution consisted of (g per liter): K_2HPO_4, 0.8; KH_2PO_4, 0.2; $CaCl_2 \cdot 2H_2O$, 0.05; $MaSO_4 \cdot 7H_2O$, 0.5; $FeSO_4 \cdot 7H_2O$, 0.01; and $(HNH_4)_2SO_4$, 1.0 dissolved in deionized water. We used a polyester polyurethane in this study. After 4 months

of incubation at a normal laboratory temperature and humidity using natural soil as an inoculum, growth in the culture was visually observed. The initial emulsion, with a uniformly white color, separated into a clear liquid phase and aggregates of polyurethane. A large population of bacteria was detected on the aggregates. In the sterile control, the uniformly white emulsion remained unchanged.

We isolated and purified two bacterial isolates from these cultures. After a series of tests, they were found to be taxonomically identical. These isolates designated as 8c-2-b and 8c-2-d are aerobic, rod shaped, Gram positive, oxidase negative bacteria. They are both capable of reducing NO_3- to N_2, and are positive for urease, esculin hydrolysis, and galactosidase. They are negative for tryptophanase, glucose fermentation, arginine dihydrolas and gelatinase. They are also capable of utilizing a range of carbon source including D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-D-glycocyamine, maltose, D-gluconate, adipate, L-malate, citrate, and phenyacetate. However, caprate was not utilized by either isolate.

In order to understand the relationship of our isolates to other bacteria, we amplified 16s rRNA genes of the bacterial isolates by PCR and sequenced part of the 16s rRNA genes for definite identification. The data showed that our bacteria are closely related to *Rhodococcus globerlus* (Figure 1). They have a 99.8% similarity with the *Rhodococcus globerlus* genes deposited in the gene bank.

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Alignment:  500 C283 8-c-2b con
 0.00 % 500 C284 8-c-2d con
 0.20 % 1513 Rhodococcus globerulus
 1.61 % 1513 Rhodococcus erythropolis
 2.80 % 1514 Rhodococcus fascians-like
 2.80 % 1514 Nocardia farcinica
 2.81 % 1514 Tsukamurella wratislaviensis
 3.02 % 1513 Rhodococcus fascians
 3.20 % 1512 Nocardia nova
 3.41 % 1514 Nocardia new species
 3.61 % 1514 Nocardia corynebacteroides
 4.40 % 1512 Nocardia otitidiscaviarum
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UPGMA Tree

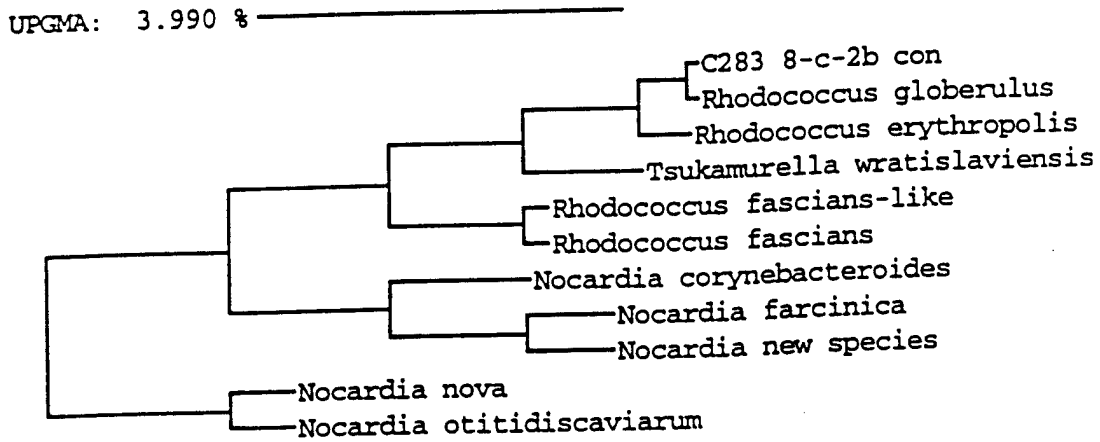

FIGURE 2. Phylogenetic tree showing the position of our isolates, from a comparisons of 16s rRNA sequences.

PERSONNEL SUPPORTED

Dr. Ji-Dong Gu

PUBLICATIONS AND PRESENTATIONS

Gu, J.-D., and R. Mitchell. 1998. Biofilm consortia responsible for degradation of polyimides and stone materials. *98th American Society for Microbiology Meeting.* 17-21 May, Atlanta,Georgia.

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